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Title: RFEF Pilot Plant for Inactivation of *Escherichia coli* in Apple Juice

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RFEF PILOT PLANT FOR INACTIVATION OF *ESCHERICHIA COLI* IN APPLE JUICE

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ABSTRACT

The nonthermal process of radio frequency electric fields (RFEF) is relatively new and has been shown to inactivate bacteria in apple juice at moderately low temperatures. However, the process has only been developed for a flow rate of 550 ml/min. The objective of this study was to scale up the RFEF technique to greater flow rates. A novel 80 kW RFEF pilot plant was designed and assembled that processed apple juice at a flow rate of 1.0 and 1.4 l/min. *Escherichia coli* K12 in apple juice was exposed to an electric field strength of 20 kV/cm at a frequencies of 21, 30, and 40 kHz. RFEF processing reduced the population of *E. coli* by 2.7 log at 60 °C and a hold time of 3 s, whereas conventional heating at the same conditions had no effect. Increasing the electric field strength and temperature, as well as decreasing the frequency, enhanced the inactivation.

INTRODUCTION

Most of the fruit juices consumed in developed countries are pasteurized using heat. Apple juice is usually pasteurized by heating to 77-88 °C in a heat exchanger and holding for 25-30 s (Moyer and Aitken, 1971). At certain conditions, the organoleptic and nutritional qualities of the juice can be damaged. Therefore, nonthermal pasteurization processes are being developed. High pressure processing (Sellahewa, 2002) and ultraviolet radiation processing (Duffy and Schaffner, 2001) have lately been commercialized, although to a limited extent. Another alternative process, that more recently has been explored, involves the use of high electric fields. In an electric field, a voltage is formed across a bacterium cell's membrane. As the voltage is increased, the opposite charges on either side of the membrane are attracted to each other with greater force and the membrane becomes thinner. Sufficiently high voltages form pores in the membrane and the cell ruptures (Zimmermann, 1986). The treatment times necessary to rupture the cells are usually less than 1 ms. The electric field also raises the temperature of the juice by ohmic heating due to the electrical resistance of the juice. The final temperatures generally are less than 70 °C and the juice is typically cooled using a heat exchanger within several seconds. The combination of lower time and temperature enables the juice to retain maximum fresh-like qualities.

High electric fields are produced by pumping the juice through a narrow gap between two electrodes and applying a high voltage. The high voltage can be applied by several different means. One method is to use direct current (DC); however, a disadvantage of this method is that charged particles in the juice may form a layer on the anode that would require periodic cleaning. Another disadvantage with DC is that undesirable electrolysis reactions may occur (Geveke, 2003; Qin et al., 1994). Using either bipolar waveforms or alternating current (AC) overcomes these problems. Bipolar waveforms are extensively used in pulsed electric fields processing where a charging power supply produces a high voltage and a high speed electrical switch delivers the stored energy to the electrodes. The power supply must then be recharged which results in pulsed processing. An AC generator continuously provides high voltage and is the source of power for radio frequency electric fields (RFEF) processing.

In previous works, RFEF processing at 30 kV/cm and 20 kHz reduced the population of *Saccharomyces cerevisiae* in water by 3.8 log (99.98 %) at 35 °C (Geveke and Brunkhorst, 2003). RFEF processing at 21 kV/cm and 55 °C inactivated *Escherichia coli* K12 in apple juice by 1.9 log (98.7 %) relative to the control (Geveke and Brunkhorst, 2004). Raising the temperature increased inactivation. Radio frequencies of 15 and 20 kHz inactivated *E. coli* better than frequencies of 30-70 kHz. The flow rate was limited to 550 ml/min by the 4 kW RFEF power supply.

The objective of this work was to design and assemble an 80 kW RFEF pilot plant system and use it to scale up the process flow rate of apple juice. The hypothesis was that the inactivation obtained at 550 ml/min could be duplicated at higher flow rates using the more powerful system.

MATERIALS AND METHODS

Escherichia coli K12 substrain C600 (Fratamico et al., 1993) was maintained on tryptose agar (Difco Laboratories, Detroit, MI) at 4 °C. The *E. coli* was cultured in brain heart infusion (Difco Laboratories) for 24 h at 37 °C. Concentrated apple juice was purchased from Tree Top (Selah, WA). The juice was diluted with water to a Brix of 12 and was inoculated from the stationary phase culture to give an approximately 6 log cfu/ml population. The solution's pH was 4.0 and its conductivity was 2.4 mS/cm.

A RFEF continuous flow pilot plant was designed, purchased, and assembled. It consisted of an 80 kW RF power supply (Scottsville, NY, model L-80) and a custom designed matching network (Ameritherm) that enabled the RF energy to be applied to a resistive load over a frequency range of 21.1 to 40.1 kHz. The maximum voltage applied was limited to 5.0 kV_{peak} in order to control the temperature rise of the apple juice.

The RFEF treatment chamber was made of Rexolite, a transparent cross-linked polystyrene copolymer (C-Lec Plastics, Philadelphia, PA). The treatment chamber was designed to converge the apple juice into a narrow flow area in order to reduce the power requirement (Geveke and Brunkhorst, 2004; Matsumoto et al., 1991; Sensoy et al., 1995). Juice entered and exited the Rexolite chamber through the annuli of cylindrical stainless steel electrodes (Swagelok, Solon, OH, part no. SS-400-1-OR) as shown in Figure 1. The electrodes were separated by a thin partition, with a channel of circular cross section through the center. The diameter and length of the channel were 0.12 cm and 0.20 cm, respectively. A 0.9 cm space between the end of each of the electrodes and the central channel prevented arcing. To increase the inactivation, the apple juice passed through two treatment chambers in series. The output of the RFEF power supply was connected to the inner electrodes between the treatment chambers and the outer electrodes were grounded. The nominal maximum electric field strength used in the study was 25 kV/cm obtained by dividing the peak voltage, 5.0 kV, by the length of the central gap, 0.2 cm. The minimum electric field strength used in the study was 15 kV/cm.

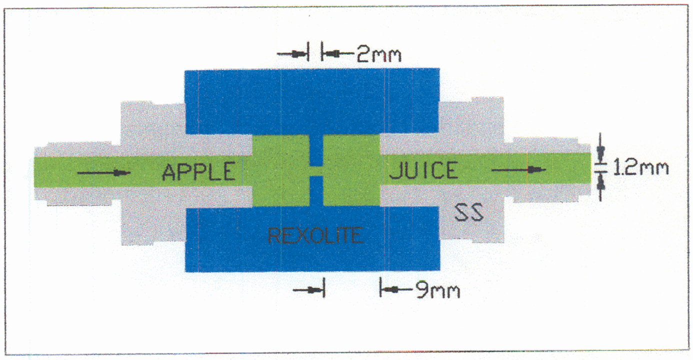

Fig. 1: Cross-section of RFEF converged treatment chamber including Rexolite insulation and stainless steel electrodes. (all figures: Geveke)

The supplied voltage and current to the RFEF treatment chambers were measured using an oscilloscope (Tektronix, Beaverton, OR; model TDS224), current probe (Pearson Electronics, Palo Alto, CA, model 411), and a voltage divider (Ross Engineering, Campbell, CA; model VD15-8.3-A-KB-A).

QuickField™ (Tera Analysis Ltd, Svendborg, Denmark, version 5.0) finite element analysis software was used to model the anisotropic electric field strength within the treatment chamber. Figure 2 presents the model's results for an electric field strength of 20 kV/cm. The apple juice flows through the electrode and enters a field-free region. It then flows into the cylindrical gap where the field is quickly raised to 20 kV/cm. The field within the gap is nearly uniform which ensures that all of the juice is treated equally. The uniformity improves the energy efficiency of the process. By minimizing the regions within the treatment chamber where the electric field is too low to inactivate bacteria and only heats the juice, approximately less than 5 kV/cm, the energy loss is minimized. Similarly, by minimizing the regions where the field is higher than needed to inactivate bacteria, the energy loss is minimized. Thus, the outlet temperature is lessened and the apple juice is not overly treated.

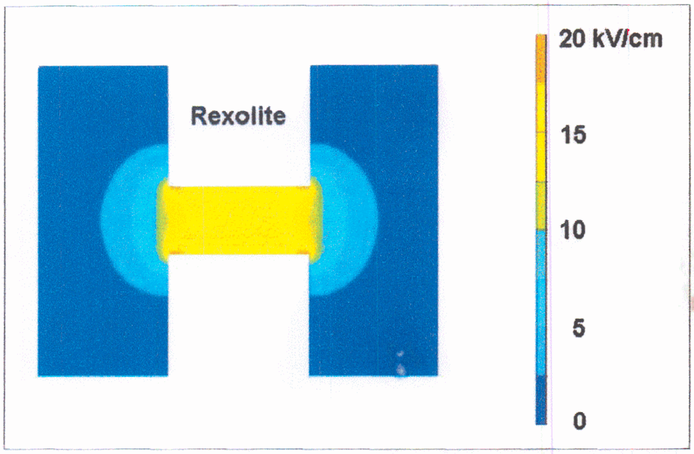

Fig. 2: Modeled anisotropic electric field strength within the treatment chamber.

The experimental system included a stainless steel feed tank and a progressing cavity pump (Moyno, Springfield, OH; model 2FG3) that supplied the apple juice to the RFEF system at a flow rate of either 1.0 or 1.4 l/min, depending on the experiment, through stainless steel tubing as shown in Figure 3. Turbulent flow within the treatment chambers (Reynolds Number > 18,000) further improved the processing uniformity. The juice was exposed to intense RFEF in the chambers for a total duration of 270 μs at 1.0 l/min and 190 μs at 1.4 l/min. The juice was exposed to 2 AC cycles of RFEF in each chamber at a frequency of 21.1 kHz and a flow rate of 1.4 l/min. The inlet temperature to the RFEF treatment chamber was controlled using a stainless steel heat exchanger (Madden Manufacturing, Elkhart, IN; model SC0004) and a temperature controller (Cole-Parmer, model CALL 9400).

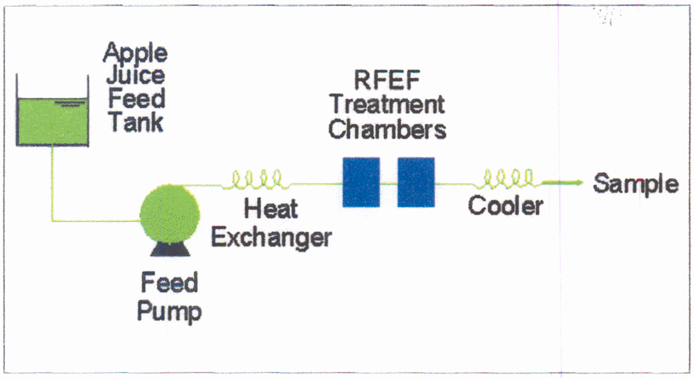

Fig. 3: Schematic diagram of continuous RFEF process.

The temperatures of the apple juice immediately before and after the RFEF treatment chambers were measured with 3.2 mm diameter chrome-constantan thermocouples (Omega Engineering, Inc., Stamford, CT). The temperatures were continuously logged to a data acquisition system (Dasytec USA, Amherst, NH, Dasylab version 5.0). The outlet temperatures ranged from 50 to 65 °C.

The apple juice was quickly cooled after exiting the treatment chamber to less than 25 °C using a stainless-steel heat exchanger (Madden Manufacturing, model SC0004). The lengths of time for the juice to travel from the treatment chamber to the sample cooler were 3 and 2 s respectively for the 1.0 and 1.4 l/min flow rates.

Controls were performed to determine the effect of temperature alone. In order to ensure that the control juice received the same time and temperature history as the treated juice, the RFEF, converged treatment chambers were replaced with an ohmic heating chamber. The chamber consisted of two stainless steel electrodes (Swagelok, Solon, OH, part no. SS-400-1-OR) inserted into a 10.2 cm length of 0.64 mm ID plastic tubing. The ohmic heater quickly brought the juice temperature up to the desired temperature. The control juice was identically held for either 2 or 3 s before cooling to less than 25 °C.

Appropriate dilutions of the product samples were plated on tryptose agar using a spiral plater (Spiral Biotech, Bethesda, MD; model Autoplate 4000) and incubated at 37 °C for 24 h. Enumerations were made with a colony counter (Spiral Biotech, model CASBA 4).

Each RFEF experiment was performed in duplicate. Results were expressed as the means of these values.

RESULTS AND DISCUSSION

The new 80 kW RFEF system successfully inactivated *Escherichia coli* K12 in apple juice at nonthermal conditions. Whereas the previous 4 kW RFEF system had been limited to a flow rate of 0.55 l/min, the 80 kW system was capable of treating 1.4 l/min. The extent of microbial inactivation is dependent on the electric field strength, treatment time, frequency and temperature.

A series of experiments were performed at 21.1 kHz to determine the effects of electric field strength and temperature on inactivation. The population of *E. coli* in apple juice was reduced by 2.2 log after being exposed to a 20 kV/cm peak electric field at a treatment chamber inlet temperature of 26 °C, treatment time of 270 μs, outlet temperature of 55 °C, and hold time of 3 s (Fig. 4). Applying the same field at an outlet temperature of 60 °C resulted in a reduction in *E. coli* of 2.7 log. When the juice was ohmicly heated at the same frequency, 21.1 kHz, to the same outlet temperature, 60 °C, and held for the same time, 3 sec, the population of *E. coli* was unaffected.

Increasing the flow rate to 1.4 l/min, and hence decreasing the treatment time to 190 μs and the hold time to 2 s, lessened the treatment effectiveness somewhat as shown in Figure 5. The population of *E. coli* in apple juice was reduced by 2.1 log after being exposed to a 20 kV/cm field at a treatment chamber outlet temperature of 60 °C. Increasing the field strength to 25 kV/cm at the same temperature resulted in a reduction in *E. coli* of 2.3 log. The nonthermal inactivation is believed to be due to dielectric breakdown of the cells (Zimmermann et al., 1974). Using a 4 kW RFEF system, *E. coli* in apple juice was reduced by 1.9 log at 55 °C, relative to the control, at a flow rate of 0.55 l/min and a hold time of 4 sec (Geveke and Brunkhorst, 2004). The results of the present study, with a uniquely designed 80 kW RFEF pilot plant, successfully scaled up the RFEF process to 1.4 l/min.

Experiments were conducted to determine the effect of frequency on inactivation. The inactivation of *E. coli* in apple juice was substantially increased as the frequency was decreased from 40.1 kHz to 21.1 kHz as shown in Figure 6. In a previous work, significantly greater inactivation of *E. coli* in apple juice occurred at frequencies of 15 and 20 kHz compared to frequencies of 30 to 70 kHz.

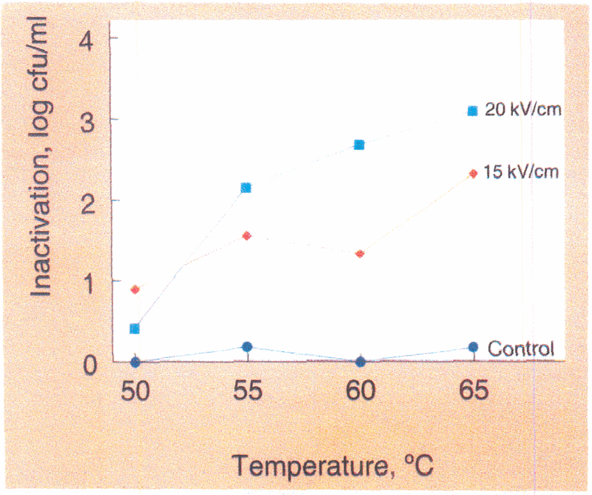

Fig. 4: Effects of temperature and electric field strength on the inactivation of *E. coli* at 270 μs RFEF treatment time and 3 s hold time (1.0 l/min flow rate). Means of two replicate experiments. ●, Control (<<1 kV/cm); ◆, 15 kV/cm; ■, 20 kV/cm.

(Geveke and Brunkhorst, 2004). These results are extremely interesting, not only because they indicate that the RFEF process could be more efficient at even lower frequencies, but also because RFEF equipment costs should be significantly less at lower frequencies as well.

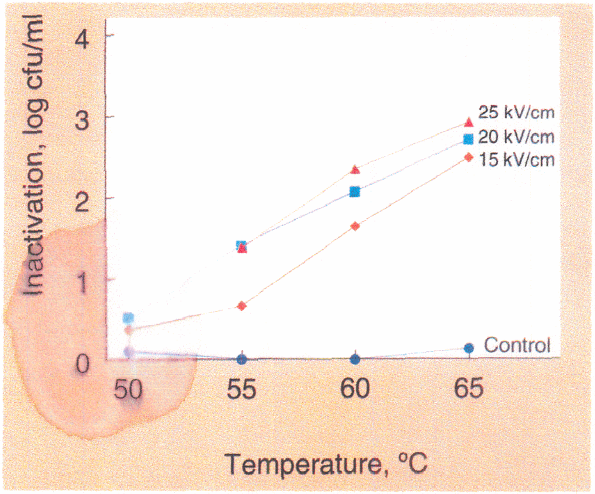

Fig. 5: Effects of temperature and electric field strength on the inactivation of *E. coli* at 190 μs RFEF treatment time and 2 s hold time (1.4 l/min flow rate). Means of two replicate experiments. ●, Control (<<1 kV/cm); ◆, 15 kV/cm; ■, 20 kV/cm; ▲ 25 kV/cm.

The energy costs of alternative pasteurization processes are an important factor in determining whether the new technologies will be commercialized. The electrical costs were calculated for the case of RFEF processing of apple juice at 15 kV/cm, 1.4 l/min and 65 °C. At these conditions, the population of *E. coli* was reduced by 2.5 log (Fig. 5). Based on the flow rate and the voltage and current measured by the oscilloscope, 3.0 kV_{peak} and 1.2 A_{peak}, respectively, the energy applied was 77 J/ml. From the inlet temperature, 50 °C, the energy calculated to raise the temperature of the apple juice to 65 °C is 63 J/ml. This is in fairly good agreement with the energy calculated using the current and voltage. The estimated energy required for a 5 log reduction using pulsed electric fields (PEF) ranges from 100-400 J/ml (Barsotti and Cheftel, 1999; Schoenbach et al., 2002). It is probable that the RFEF electrical costs for a 5 log reduction will be similar to those of PEF as they are both considered electroporation processes (Geveke and Brunkhorst, 2004). Based on the U.S. Department of Energy's data for the average industrial electric price for the first nine months of 2003 of $0.050/kWh, the energy cost for the RFEF process was 0.11¢/l of apple juice. For comparison, the energy costs for conventional thermal pasteurization, with heat regeneration or recovery, is 0.05¢/l or less.

Additional studies are recommended. The RFEF process needs to be further scaled up to be of commercial inter-

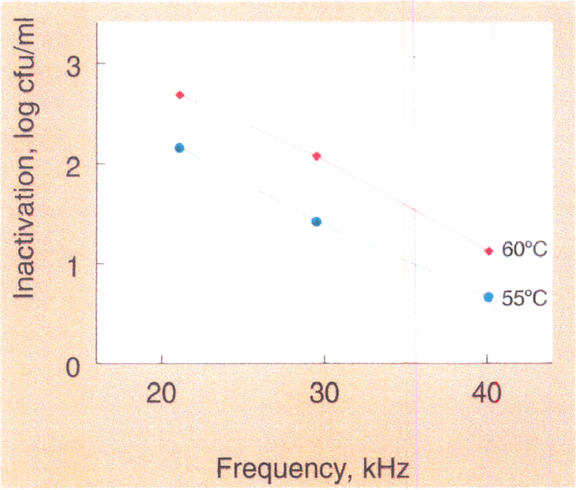

Fig. 6: Effect of frequency on the inactivation of *E. coli* at 20 kV/cm, 270 μs RFEF treatment time and 3 s hold time. Means of two replicate experiments. ●, 55 °C and ◆, 60 °C.

est. In addition, a 5 log reduction is desirable. This should be achievable by adding several more treatment chambers in series. Finally, RFEF processing at much lower frequencies, where the efficiency may be enhanced, deserves attention.

CONCLUSIONS

The radio frequency electric fields (RFEF) process can be successfully scaled up from 0.55 l/min to 1.4 l/min using an innovative pilot plant consisting of an 80 kW power supply and novel matching network. Nonthermal inactivation of *Escherichia coli* K12 in apple juice can be obtained and is dependent upon the electric field strength, frequency, treatment time and temperature. The RFEF process should be capable of pasteurizing vegetable and fruit juices at moderately low temperatures by increasing the number of treatment stages.

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